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MORPHOLOGY OF STAMENS AND STAMINODIA OF
ECHINOCYSTIS LOBATA (Michx.) T. & G.

By

Engracia B. Arguelles

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science at South Dakota
State College of Agriculture
and Mechanic Arts

August 1956

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**MORPHOLOGY OF STAMENS AND STAMINODIA OF
ECHINOCYSTIS LOBATA (Michx.) T & G**

This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

ACKNOWLEDGEMENTS

I wish to take this opportunity to express my gratitude to Dr. Ward Miller, Head of Botany Department, for the suggestion of this problem and for the help he gave during the course of this research.

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INTRODUCTION

The number and nature of stamens in flowers of the Cucurbitaceae has long been a controversial problem among students of floral morphology. Haudin 1885, according to Chakvarty (2), thought that 3 was the basic number of stamens in this family. Haudin imagined a 2-2-1 stamen arrangement where 2 is a complete stamen (tetrasporangiate), and 1 is a half-stamen (bisporangiate). In the few species of this family which have five stamens, he believed that the two complete stamens split resulting in what he called four half-stamens. These, together with the normal half-stamen, would account for the total of five, each bearing two sporangia.

Payer 1857, according to Miller (10), "presented the opposing view, that the small bisporangiate stamen was normal, while the large tetrasporangiate stamen had resulted from the union of two normal ones". Miller cites other investigators through the latter half of the nineteenth century, whose papers were not available to this writer. These workers, however, sided either with Haudin or Payer in their interpretations.

More recently Reimlich (6), in his study of the flowers of cucumber, agreed with Haudin's earlier work, calling one stamen of the flower a half-stamen. In 1928 Miller (10) investigated the staminate flower of Echinocystia lobata, and he came to close agreement with Payer. Miller found that there are only two stamens in the staminate flower of this species; one tetrasporangiate and one bisporangiate. But he showed the evidence that there are fundamentally three stamens, each bisporangiate; but that two of them become united during their

early development, appearing finally as one tetrasporangiate stamen. Chakvarty (2) who has most recently worked on the stamen situation in Cucurbitaceae, agreed with Miller's interpretation, and suggested further that reduction from pentamery to trimery in the family was due to fusion. He did not accept the half-stamen idea either.

In the foregoing investigations of cucurbitaceous stamens, little attention has been given to the staminodia in the pistillate flowers. With a suspicion that the staminodium situation might reveal some primitive relationships among stamens, this present investigation of staminodia was undertaken. Once the matter is cleared up, it should contribute to the working knowledge of both taxonomists and morphologists.

Moreover, study of the nature and degree of development of staminodia in Echinocystis lobata may prove helpful to our understanding of the separate sexes among many flowering plants. Literature does not mention that the pistillate flower of Echinocystis lobata may produce pollen grains, but it should be mentioned that in some pistillate flowers the author did find functioning "stamens" with apparently normal pollen.

In his studies on sex balance in squash, Nitsch (11) found that more pistillate flowers were produced at low temperatures than at high temperatures; and that artificially increasing the length of day, consistently retarded production of pistillate flowers, so that the sex balance swayed toward maleness. Since the flower specimens for the present investigation of Echinocystis lobata were collected in early September, when days were still long and temperatures still high, the occurrence of occasional functioning stamens in pistillate flowers

may well be another instance of the trend toward maleness which Mitsch describes. A study of the effect of light and temperature on the development of stamens in the pistillate flower of Echinocystis lobata, would be a promising problem for physiologists.

MATERIALS AND METHODS

The flowers of Echinocystis lobata were collected September 9, 1955, along a road at Lake Campbell, near Brookings, South Dakota. The flowers were fixed in formalin-acetic-alcohol. Following fixation, parts of the perianth were cut off to facilitate the removal of air by a vacuum pump. Specimens were then washed in 50% ethyl alcohol, dehydrated and embedded in paraffin. Both transverse and longitudinal sections were cut at 8 microns, and stained with safranin and fast green. Drawings were made with the aid of a camera lucida.

INVESTIGATION

a. Habit

Pistillate flowers are borne singly, rarely two together, in the same axil where staminate flowers are borne. The former are short-stalked and epigynous. The staminate flowers are in an inflorescence which is either racemose or paniculate. The pistillate flowers are more often yellowish or greenish whereas the staminate flowers are nearly white. The pistillate hypanthium is covered by spines which, when dried at maturity, are sharp and prickly to the touch. The ovary is ovate and with either 2 or 4 locules, each enclosing 2 ovules. Arising from the hypanthium is a campanulate perianth-tube, bearing 6, rarely 7, lanceolate corolla lobes and as many calyx lobes. The latter are slender, pointed

at the apex and cylindrical at the base. They are borne alternately with the lobes of the corolla.

The three staminodia arise at a level of the perianth above the ovary, their filaments united with the base of the style and their anthers more or less free and curved. Figure 1 is used to show a longitudinal section of a pistillate flower, and to show the relation of the staminodia to other parts. In nature the staminodia are hard to find by an unaided eye, owing to their small size, and because the over-arching lobes of the stigma hide them from view. Engler and Prantl (4) described them as hair-like; Rydberg (12), as more or less reduced. The anthers are usually curved, due to the downward folding of the two lobes of the stigma. The filament is flat and is nearly as wide as the anther, except that the latter narrows towards its apex. It is important to note that two staminodia share the available space between two stigmatic lobes on one side, whereas the third staminodium is borne singly between the two lobes on the opposite side. The two staminodia that grow close together, sharing the available space between two stigmatic lobes, have been found to unite in such a manner that their adjacent sides are continuous, due to meristematic activity in the intervening receptacle tissue, so that they have grown upward in apparently a single unit, except for the bent parts of the anthers (fig. 2). Figure 3 is used to show how the adjacent sides are so completely united. When such union occurs, only two staminodia are found, just as two stamens are found in the staminate flower; and the stamen which has resulted from the union is tetrasporangiate. Miller (10) has advanced this theory of union or continuity, due to close growth of

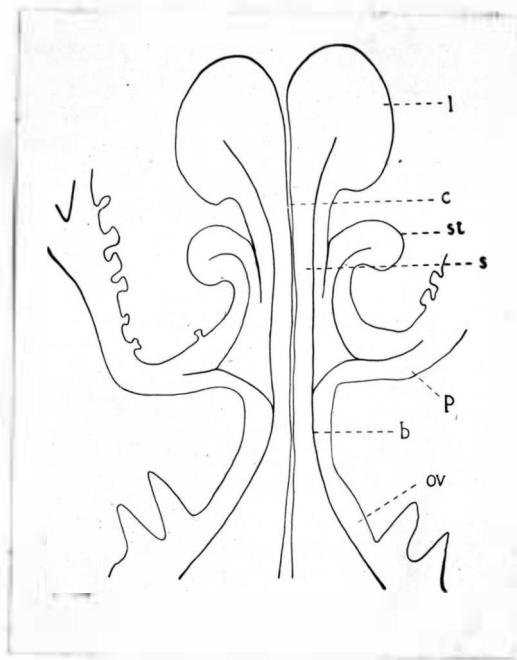


Figure 1. Longitudinal section of the upper part of the pistillate flower: b, vascular bundle; c, stylar canal; l, stigmatic lobe; ov, ovary; p, perianth; s, style; st, staminodium; x 25

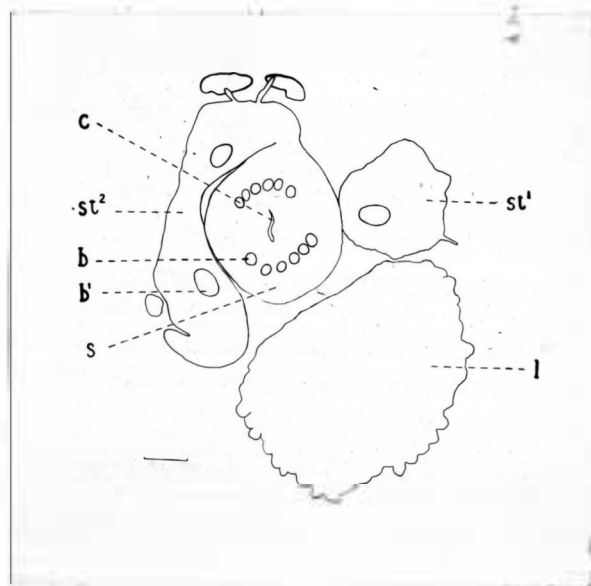


Figure 2. Cross section of the pistillate flower at the level of the filaments of staminodia: b, vascular bundle of style; b¹, vascular bundle of staminodium; c, stylar canal; l, stigmatic lobe; s, style; st¹, single staminodium; st², double staminodium; x 55

the two staminodia. He believed that the close proximity of the two staminodia in the pistillate flower may suggest the union that takes place between the two stamens which form the usually tetrasperangiate stamen in the staminate flower. However, he did not see two staminodia actually grow together as a single unit.

b. Vascular Supply

A detailed study of the vascular supply to staminodia shows that they are related to the sepal bundles. Starting from the pedicel, serial cross sections show that 4 large bundles supply the hypanthium, ovules and spines. The hypanthium continues into a slender tube as it rises above the ovary. At the level immediately below the expansion of the perianth (fig. 1), a cross section (fig. 4) shows twelve bundles entering the upper part of the flower, i.e. the perianth and style. Each of these twelve bundles divides radially (some bundles divide much earlier than others) into two bundles. Consequently, after the branching, two sets of bundles are formed: an inner set and outer one, each set with twelve bundles. The former set enters the style of the pistil while the latter becomes the perianth bundles (fig. 5). Six of those that enter the perianth extend into the six petals where they become the mid-bundles. The remaining six become the mid-bundles of the six sepals. The outer set is so distributed that the bundles which supply the sepals alternate with those which supply the petals.

The twelve bundles that supply the perianth not only become mid-bundles of the respective organs but also exhibit different habits

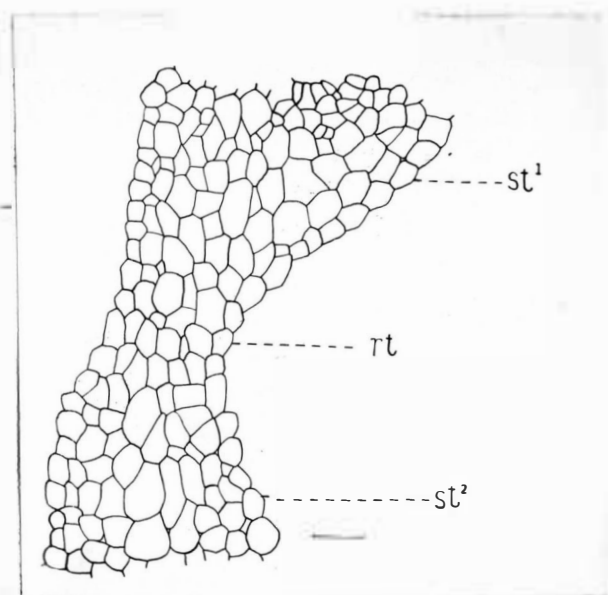


Figure 3. Cross section through union of two staminodia: rt, receptacular tissue; st¹, single staminodium; st², single staminodium; x 322

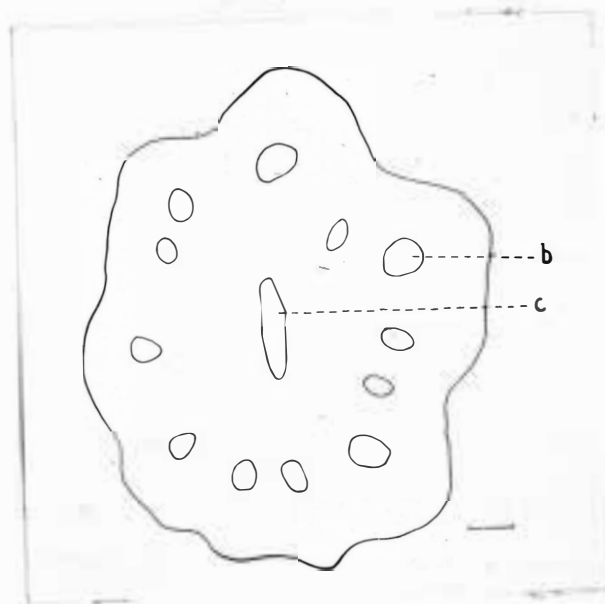


Figure 4. Cross section through hypanthium below perianth tube: b, vascular bundle; c, styler canal; x 60

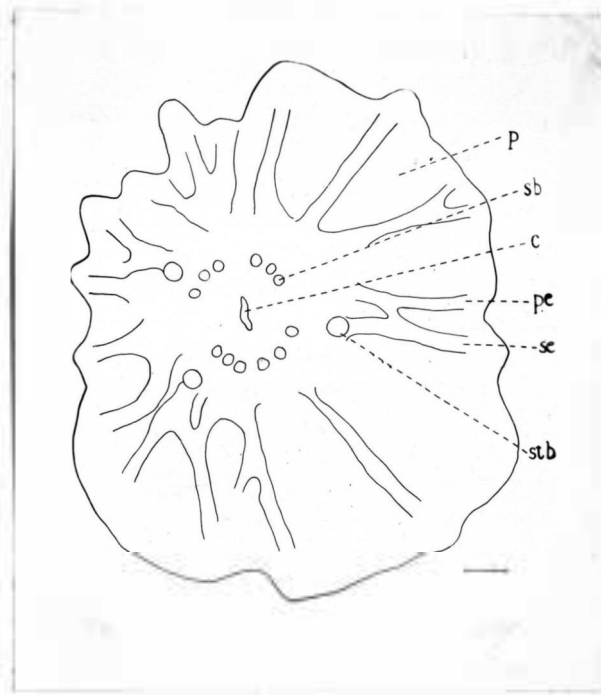


Figure 5. Cross section through base of perianth tube: c, stylar canal; p, perianth tube; pe, vascular supply to petal; sb, vascular supply to style; se, vascular supply to sepal; stb, vascular supply to staminodium; x 55

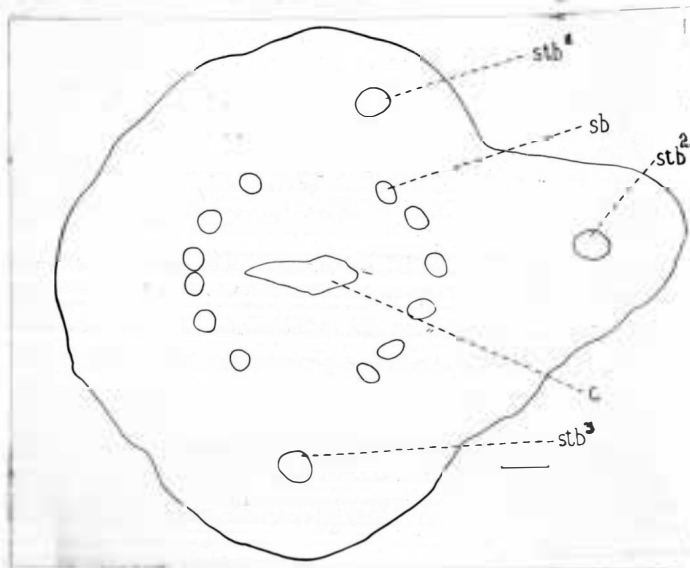


Figure 6. Cross section through base of style: c, stylar canal; sb, vascular bundle of style; stb, vascular bundle of staminodium; stb¹, vascular bundle of staminodium; stb², vascular bundle of staminodium; x 123

of branching and give rise to marginal veins of the petals. Such branching occurs before a bundle enters its respective organ, namely the sepal or petal. As a result, the main bundles and their branches appear to anastomose (fig. 5). A bundle that is destined to supply a sepal produces either 2 or 3 branches. If 3 branches are produced, two become the marginal veins of the neighboring petals, one on either side, while the third branch comes off directly in front of the branching sepal bundle, and extends perpendicularly to supply a staminodium. Thus, one branch from each of three sepal bundles supplies a staminodium. As in figure 6, the serial sections show that, at the level of the base of the style and above, the three staminodium bundles are distinct from the bundles of the style. Three separate vascular bundles supplying the staminodia force one to the conclusion that the number of staminodia is basically three, despite the fact that two of them may occasionally unite as a result of their proximity in the flower.

In all pistillate flowers examined there were basically three staminodia, though in some the two in close proximity had united. Except for this single feature of uniformity in number, however, the staminodia of different flowers showed a high degree of variability. Their shape can be modified considerably by the lobes of the stigma which curve outwards and downwards from the style, obstructing the upward elongation of the staminodia and causing them to bend around and downwards toward their own bases. Also, staminodia showed varying degrees of development, even in flowers of equal maturity.

The simplest type of staminodium found in fully opened flowers was hair-like in shape, and so small as to escape notice unless a lens

was used. The vascular supply to such a staminodium was never seen to have matured beyond procambium stage; and in no instance did this type of staminodium show evidence of sporangium development (fig. 7). This is the type described by Engler and Prantl (4) as being hair-like.

Most of the staminodia, however, had developed a bit farther than this before their progress was stopped: they were larger than the hair-like ones, especially in width. Each was traversed by a vascular bundle which, for about the first (proximal) half of its length had matured into xylem and phloem tissues; only the distal half of the bundle remained as immature procambium (fig. 8). In staminodia of this type two conspicuous cavities had developed, one on either side of the wide connective. Each cavity was seen to be preceded, in an immature staminodium as in figure 9, by periclinal divisions of the hypodermal layer, just as in the development of a microsporangium wall. Eventually the inner ones of these wall cells broke down and were apparently resorbed, leaving the empty cavity (fig. 10). No microspore mother cells were differentiated and therefore no microspores were produced. But because these cavities developed where microsporangia would be expected in a stamen, because their initial development resembled that of microsporangia, and because a few staminodia did actually develop microsporangia, the latter is what these cavities are interpreted to be.

In a few pistillate flowers the three staminodia had matured still farther. They were not only large, with well developed vascular bundles, but were actually stamens with either pollen mother cells or pollen grains enclosed (figs. 11, 12). In this type, however, there were variations in number of microsporangia. The simpler ones were

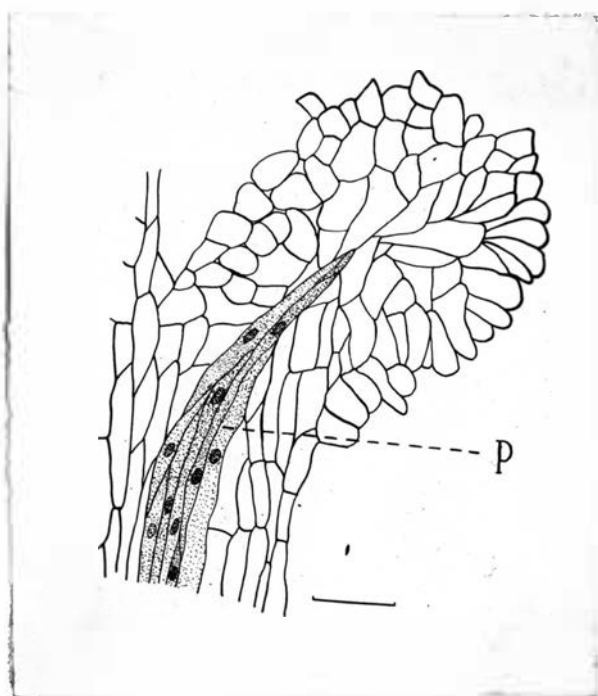


Figure 7. Longitudinal section of hair-like staminodium: p, procambium; x 215

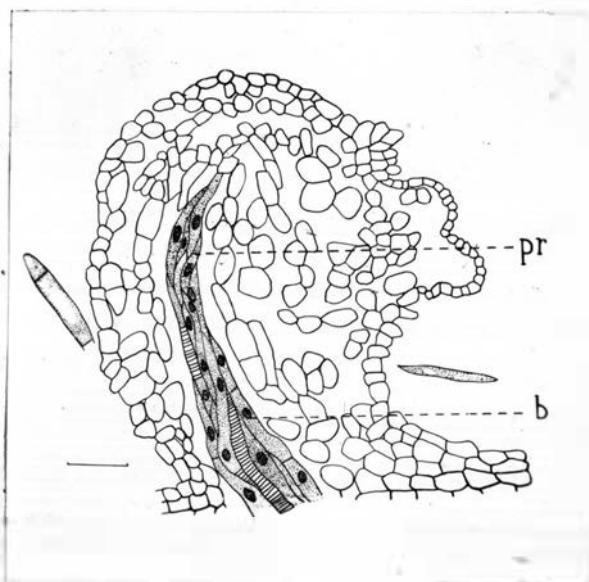


Figure 8. Longitudinal section of a mature larger staminodium: b, mature vascular bundle; pr, procambium; x 164

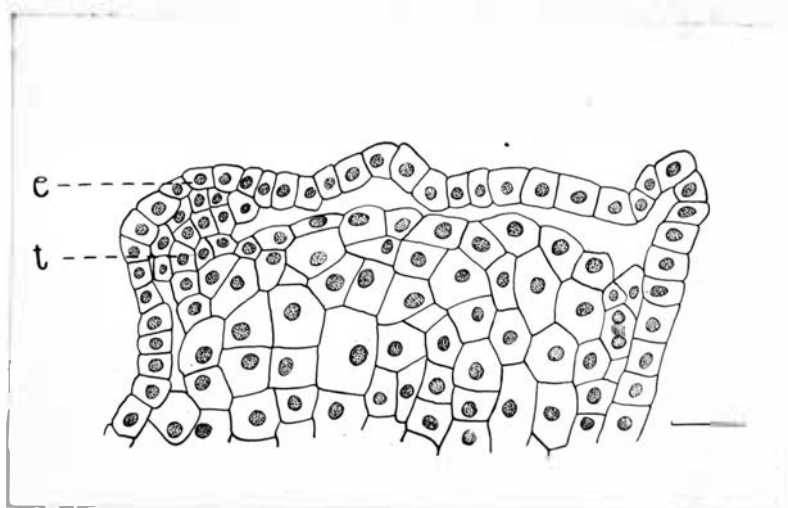


Figure 9. Cross section of young larger staminodium: e, epidermis; t, sporogenous tissue; x 455

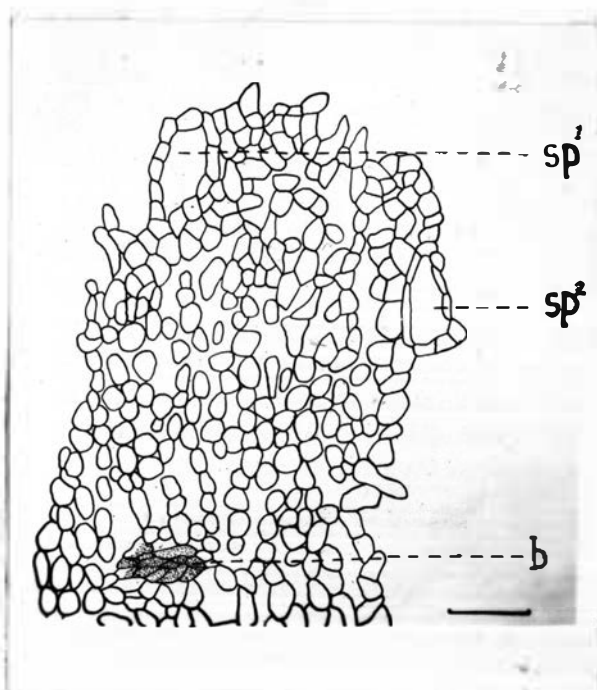


Figure 10. Cross section of mature larger staminodium: b, vascular bundle; sp^1 , sporangium; sp^2 , sporangium; x 200

bisporangiate (fig. 12), i.e. they bore but two microsporangia. In such a stamen two rows of microspore mother cells would be found in each of the two sporangia. They would be found lying side by side, without intervening sterile tissue (fig. 12). The failure of sterile tissue to develop between contiguous rows is evidently the reason why such a stamen was bisporangiate instead of tetrasporangiate. A single cross section to show the two sporangia together is impossible because they develop at different levels on the stamen. If the two sporangia were continuous with each other, they would constitute the S-shaped figure which Miller (10) describes for the staminate flower; but they were not found to unite at any point. It would appear, then, that two long, slender sporangia, lying side by side, had become one by failure of the intervening wall to develop; and then that this one long sporangium had become two short ones by the sterilization of potential sporogenous tissue at the middle of the long, S-shaped strip.

Even the usual tetrasporangiate stamen bearing 4 microsporangia was found. In this type the sterile wall between the two parallel rows of mother cells was completed (fig. 11), and four microsporangia were formed instead of two. Hence either of two types of tetrasporangiate stamen may occasionally be found, one which is the result of union of two bisporangiate stamens, and the other which results from formation of a sterile wall between the two rows of mother cells.

c. Microsporangium and Pollen

Beneath the anther's epidermis, microsporangium initials (fig. 13) cut off parietal cells that develop the three-layered wall of the

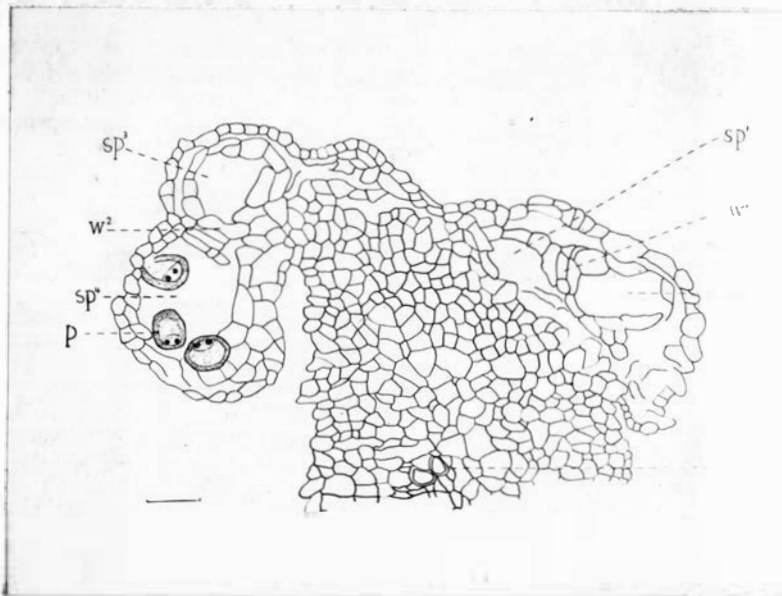


Figure 11. Cross section of a tetrasporangiate stamen: b, vascular bundle; p, pollen grain; sp¹, sporangium; sp², sporangium; sp³, sporangium; sp⁴, sporangium; w¹, sterile wall; w², sterile wall; x 144

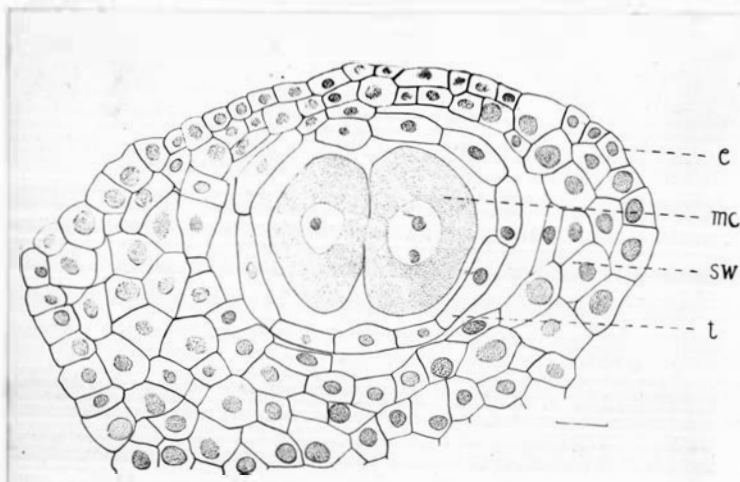


Figure 12. Cross section of a young bisporangiate sporangium: e, epidermis; mc, mother cells; sw, sporangium wall; t, tapetum; x 615

sporangium, the innermost layer of the three becoming the tapetum (fig. 12). Next inside the tapetal layer are the large microspore mother cells. In the staminate flower (figs. 14, 15), every stamen examined had its mother cells consistently in uniseriate rows, one row in each sporangium. In the sporangium of bisporangiate stamens of the pistillate flower however, mother cells commonly occur in two rows (fig. 12). This fact suggests that, if two rows of sporangium initials differentiate close enough together in the young anther, the sterile tissue between them, if present at all, may be too meager to continue as a separating wall. Consequently, the mother cells of two sporangia come to be included in a single sporangial cavity.

Microspore mother cells round off from one another prior to their reduction divisions, just as Kirkwood (8) described them in the staminate flower. Also in accord with his findings, these rounded mother cells undergo considerable contraction and distortion (fig. 16); then they divide to form tetrads of microspores. So far as one can tell from developmental phenomena, and from final structure, the microspores produced in pistillate flowers are perfectly normal and viable. Their wall and protoplast features compare with those of microspores produced in staminate flowers, even to the active nucleus with its nucleolus (fig. 17). No pollen grains from a pistillate flower were germinated, however, to prove their viability. Pollen grains are shed by the disorganisation of the sporangium wall cells and subsequent rupture of the epidermis.

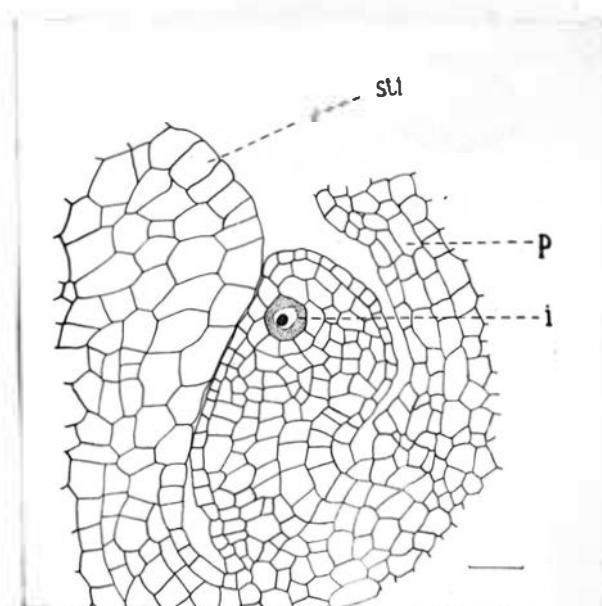


Figure 13. Longitudinal section of a stamen primordium: i, sporangium initial; p, perianth; sti, stigmatic lobes; x 329

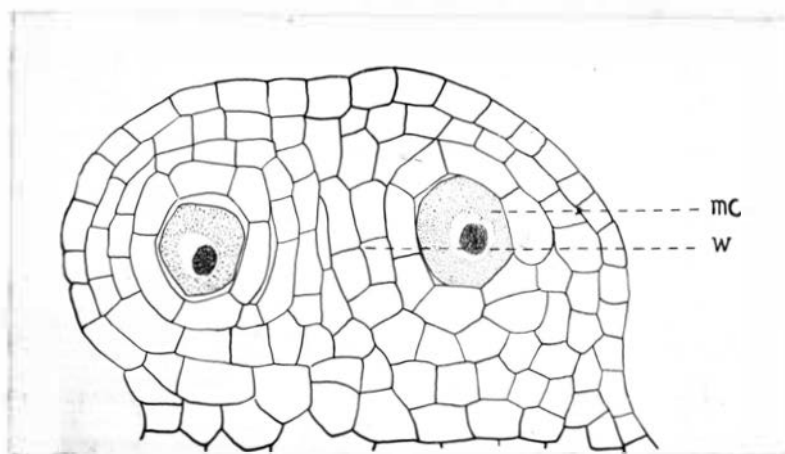


Figure 14. Cross section of two young sporangia at mother cell stage of a staminate flower: mc, mother cell; w, sterile wall; x 980

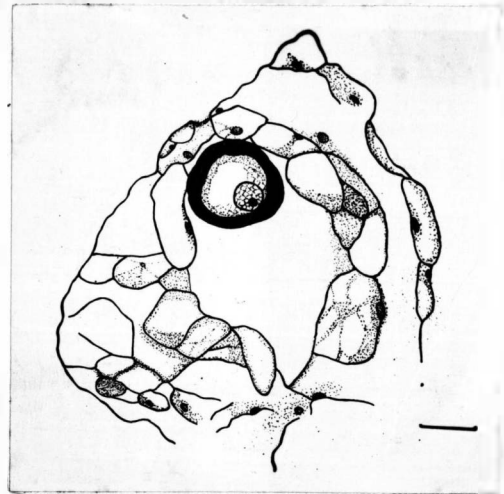


Figure 17. Mature pollen in a sporangium: x 309

Figure: 15. Longitudinal section of sporangia of staminate flower at mother cell stage: e, epidermis; mc, mother cell; sw, sporangium wall; t, tapetum; w, sterile wall; x 322

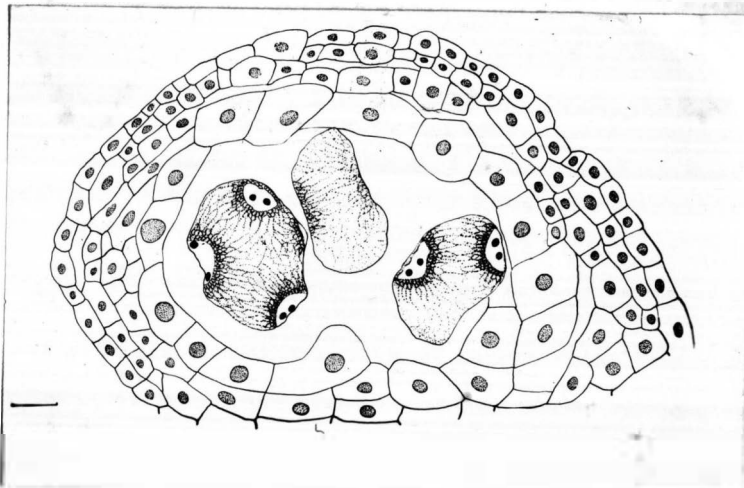


Figure 16. Tetrads in sporangium: x 700

DISCUSSION

Flowers of the Cucurbitaceae bear both tetrasporangiate stamens and bisporangiate ones; but interpreting the former as whole stamens merely because they bear four sporangia, and the latter as half-stamens merely because they bear two, is illogical. Throughout the ferns, the number of sporangia borne by sporophylls is variable; but a sporophyll is a whole sporophyll whether it bears few sporangia or many. Similarly, flowering plants exhibit variability in number of sporangia per stamen. The fact that four is the number most frequently developed on a stamen cannot be taken as proof that a stamen is complete only if it has four, that it is a half-stamen if it has two, or a fourth-stamen if it has but one. Nor can it be interpreted as a poly-stamen just because it happens to bear more than four sporangia. Wylie, according to Maheshwari (9), reports that stamens of Elodea canadensis regularly produce two sporangia each, with no suggestion of a greater number at any stage. This is also the situation in the Asclepiadaceae. Caldwell (1) describes the stamen of Lupinus minor as producing a single sporangium which, late in development, becomes subdivided by sterile tissue into four locules. Maheshwari (9) refers to a paper by Witner 1937, who found that, in Vallisneria, there are all gradations from unilocular to tetralocular stamens, depending upon the degree of development of sterile plates through the sporogenous tissue. Such variability resembles in principle what the present writer found in staminodia in Echinogystia. More than the usual four sporangia have been reported from Orchidaceae and Conagra-ceae by various investigators. Coulter and Chamberlain (3) refer to a paper by Van Tieghem as early as 1895, who described the number of pollen

sacs in stamens of the Loranthaceae as being exceedingly variable, ranging from one to an indefinite number. And Johnson (7) found that the stamens of Piper betle may have four, three, or two sporangia, or only one.

A stamen character more significant morphologically than mere number of sporangia is the vascular supply. One vascular bundle to a stamen is so nearly universal among flowering plants that any number other than one immediately invites investigation. The number of vascular bundles supplying a stamen, or a stamen complex, is therefore a more dependable index of the basic number of units in such a complex than is the number of externally visible parts. More specifically, if what appears to be a single stamen receives two vascular bundles, an investigator immediately suspects a union, more or less complete, between two units. Such a union was found to be a fact, as stated above (fig. 2), among staminodia of Echinocystis. Three vascular bundles clearly indicate three staminodia, even though two staminodia have united completely enough to appear externally as one. Miller (10) suspected this from his study of the staminate flower, and the present study in pistillate flowers supports that conclusion. The two-stamen flower in Echinocystis has not one complete stamen and one half-stamen; but rather two stamens, one of which is single and the other double. Heimlich's complete stamen and half-stamen in the flower of cucumber may need re-investigating in this connection.

The variations in number of staminodia, in number of micro-sporangia, and in level of development in the latter, all suggest a degree of fluctuation that might well be expected in an organ, or set of

organs, that is undergoing phylogenetic degeneration. Under suitable conditions, staminodia actually revert to stamens with 2 or 4 microsporangia and produce pollen that appears to be viable. On the other hand, unsuitable conditions result in staminodia that are sterile, and hair-like in appearance. If, as it would appear, a separation of the sexes into different flowers is evolving out of a condition of bisexuality in flowers, obviously the staminate flower has progressed farther in losing evidences of the opposite sex than the pistillate flower has; for while the former exhibits no external evidence of carpels, the latter does exhibit staminodia, some of which occasionally produce pollen.

SUMMARY

The purpose of this investigation was to examine staminodia in the pistillate flowers of Echinocystis lobata with the hope of finding details of development and final structure which would contribute to an understanding of the stamen situation in the staminate flower. It is not uncommon to find two of these uniting into one unit structure, during their growth, if their primordia arise in close proximity to one another. This feature, together with the three-bundle vascular supply, indicates clearly that there are basically three staminodia in the pistillate flower, and it indicates as well that the two stamens in a staminate flower are fundamentally three complete stamens.

It is of importance that staminodia sometimes become functional stamens, and that this reversion to a possible ancestral condition appears to be a response to environmental conditions which accentuate maleness in the flower.

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